Studies on Neurotensin I. Effects on Gallbladder Motility

AUTHOR(S):

FUJIMURA, MASAKI

CITATION:

FUJIMURA, MASAKI. Studies on Neurotensin I. Effects on Gallbladder Motility. 日本外科宝函 1989, 58(5): 405-413

ISSUE DATE:

1989-09-01

URL:

http://hdl.handle.net/2433/203901

Arch Jpn Chir 58(5), 405～413, Sept., 1989

Studies on Neurotensin. I. Effects on Gallbladder Motility

Masaki Fujimura

Second Department of Surgery, Shiga University of Medical Science
Received for Publication, June 6, 1989

Summary

Effects of neurotensin (NT) on gallbladder contraction were examined both in vivo and in vitro. Cholecystokinin-octapeptide (CCK-8) was used to evaluate the methods used in this study and to compare the action of NT on the gallbladder. In *In vivo studies*, gallbladder contraction was monitored by strain-gauge force transducers implanted on the surface of the dog gallbladder. Bolus intravenous (IV) injection of NT at doses of 20 and 40 ng/kg caused gallbladder contraction of similar of magnitudes in terms of contractile force, while CCK-8 caused contraction dose-dependently. Continuous IV infusion of NT at doses of 250 and 500 ng/kg/hr, which resulted in an elevation of blood levels of NT comparable with those achieved by endogenous release, induced a transient gallbladder contraction. Both maximum contractile force and onset time of contraction were similar to both does of NT. In contrast, CCK-8 induced gallbladder contraction was sustained during infusion of CCK-8 and was dose-dependent for both maximum contractile force and onset time of contraction. NT-induced gallbladder contraction was completely abolished by atropine treatment. In *In vitro studies* of longitudinal rabbit gallbladder muscle strips, NT was ineffective, while CCK-8 caused a dose-dependent contraction. The present study shows that NT can stimulate gallbladder contraction in the dog via cholinergic pathways.

Introduction

Neurotensin (NT) was discovered by Carraway and Leeman in 1973[8] as a by-product during purification of substance P from bovine hypothalamus. It was found to produce vasodilation and hypotension when injected into rats. The sequence of NT (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH; MW=1673), its synthesis and a specific radioimmunoassay were reported shortly thereafter[9,10,11].

Although initially found in the brain, over 90 percent of NT in the body occurs outside the central nervous system, largely in the small intestine[12]. There are detectable levels in the esophagus, stomach, duodenum, and colon. However, the vast majority of NT is found in the

Key words: Neurotensin, Cholecystokinin-8, Gallbladder Contraction, Rabbit Gallbladder Strip.
索引語：ニューロテンシン，コレシストキニン-8，胆嚢収縮，ウサギ胆嚢筋条片
Present address: Second Department of Surgery, Shiga University of Medical Science, Seta, Otsu-city, 520-21 Japan.

small intestine, with the greatest amount in the ileum of rats, dogs[14,15,21,26,42] and humans[17,27].

In the small intestine of man and animals, NT exists not in the nervous system but in mucosal cells (called N cells), which are typical gut endocrine cell-type with basal granules and microvilli reaching into the gut lumen. From these morphological characteristics of the N cells, it is thought that NT is released into the blood stream by suitable luminal stimulants (such as fatty nutrients[31]).

Gastrointestinal effects of NT include mesenteric vasodilation[30], inhibition of gastric acid secretion[3,5,25], alteration of gastrointestinal motility[29,32] and pancreatic exocrine secretion[22,35,36]. The effect of NT on gallbladder function has been reported to increase intragallbladder pressure in chronic fistula dogs[33], but effects on gallbladder motility are not elucidated. The purpose of this study was to examine the effect of NT on gallbladder contraction both in vivo and in vitro.

Materials and Methods

In vivo study

Five mongrel dogs of both sexes (18–22 kg) were prepared with strain-gauge force transducers[18,19] (provided by Dr. ZEN ITOH, Maebashi, Japan). Under secobarbital anesthesia (30 mg/kg body weight, IV), force transducers were sutured on the midportion of the serosal surface of the gallbladder with a 4–0 atraumatic needle in a direction to detect the contractions of the circular muscle. The lead wires from this transducer were directed to the hepatic hilus along the cystic duct and lightly sutured on the gallbladder surface at approximately 2 cm distal from the sensor. In addition, a bipolar electrode was implanted onto the surface of the duodenum. The lead wires were exteriorized at the right subcostal margin, passed through a subcutaneous tunnel, pulled out through a stab wound made between the scapulae, and sutured to the skin. After the operation, small connectors were attached to the lead wires and the dog was fitted with a canvas jacket to protect the wire terminals.

Studies were started three weeks after the above operations. The dogs were fasted overnight for 18 hours with free access to water before each experiment. During each experiment, the dogs were placed in PAVLOV stands and the canvas jackets were removed. The contractile activity of the gallbladder and myoelectric activity of the duodenum were recorded with a Beckman R-611 Dynograph (Beckman Instruments Inc., Palo Alto, CA). The duodenal electrode was used to monitor electrical activity in the duodenum. While the gallbladder and duodenum were in the quiescent phase, a bolus injection of NT (10, 20 or 40 ng/kg), cholecystokinin-8 (CCK-8) (0.5, 1, 2, 3, 4 or 5 ng/kg), a continuous infusion of NT (125, 250, 500 or 1,000 ng/kg/hr) or CCK-8 (25, 50 or 100 ng/kg/hr) was given intravenously.

The continuous infusion of NT and CCK-8 was repeated during IV atropine (Invenex®, The Dexter Corp., Chagrin Falls, Ohio) treatment (25 ng/kg bolus, followed by 20 ng/kg/hr infusion).

In vitro study

Gallbladder contraction was measured by a modification[23] of the bioassay for CCK described earlier by BERRY and FLOWER[4]. Longitudinal gallbladder strips (3–4 cm long, 0.3 cm wide) were obtained from five 24-hour fasted New Zealand white rabbits (2–3 kg). The gallbladder strip was placed in an organ bath containing 20 mg of Krebs solution. Tension changes were monitored by an isometric force-displacement transducer (UC2, Gould Statham Instruments Inc., Oxnard, CA) and recorded with a Beckman R-611 Dynograph. Measurements were started after a 2–3 hour period of stabilization. The transducers were set to zero. Gallbladder strip tension in response to CCK-8 (1 ng/ml) as a control and NT (5, 10, 20 or 40 ng/ml) were tested.

Solutions

NT (Bachem, Torrance, CA) and CCK-8 (Bachem, Torrance, CA) were dissolved in a concentration of 1 mg/ml in distilled water containing 0.1% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO); they were divided into aliquots and stored at −20°C until used.

Measurement and Analysis of Data

The contractile force of the gallbladder in vivo was measured in grams. In vitro tension changes of the gallbladder strip were measured in milligrams. Values are expressed as mean ±SEM. A P value <0.05 was considered statistically significant.

Results

In vivo study

IV bolus administration of CCK-8 (0.5, 1, 2, 3, 4, 5 ng/kg) caused a dose-related contraction of the gallbladder (Fig. 1, upper panel). IV bolus administration of NT (10, 20, 40 ng/kg) induced gallbladder contraction (Fig. 1, lower panel). The minimum dose of NT to cause gallbladder contraction was 20 ng/kg. Contractile force obtained by 20 ng/kg (12 pmol/kg) and 40 ng/kg (24 pmol/kg) of NT were almost the same, comparable to that obtained by CCK-8 at a dose of 2 ng/kg (1.8 pmol/kg). Continuous infusion of NT (250, 500, 1,000 ng/kg/hr) caused gallbladder contraction. The response of the gallbladder was not dose-related and transient (Fig. 2). Atropine (25 ng/kg bolus, 20 ng/kg/hr infusion) completely abolished the effect of NT on gallbladder contraction (Fig. 2, lower right panel). Continuous infusion of CCK-8 (25, 50, 100 ng/kg/hr) caused gallbladder contraction in a dose-dependent manner (Fig. 3). The gallbladder contraction induced by CCK-8 was sustained during the infusion and returned quickly to baseline after cessation of the infusion of CCK-8. Atropine diminished gallbladder contractile force (Fig. 3, lower right panel).

Summarized data calculated as contractile force at maximum contraction and onset time of contraction during NT (250, 500 ng/kg/hr) or CCK-8 infusion (25, 50 ng/kg/hr) are shown in Table 1. NT stimulated gallbladder contraction, but the contractile force or the onset time of gallbladder contraction was not significantly changed by increasing the dose of NT from 250 ng/

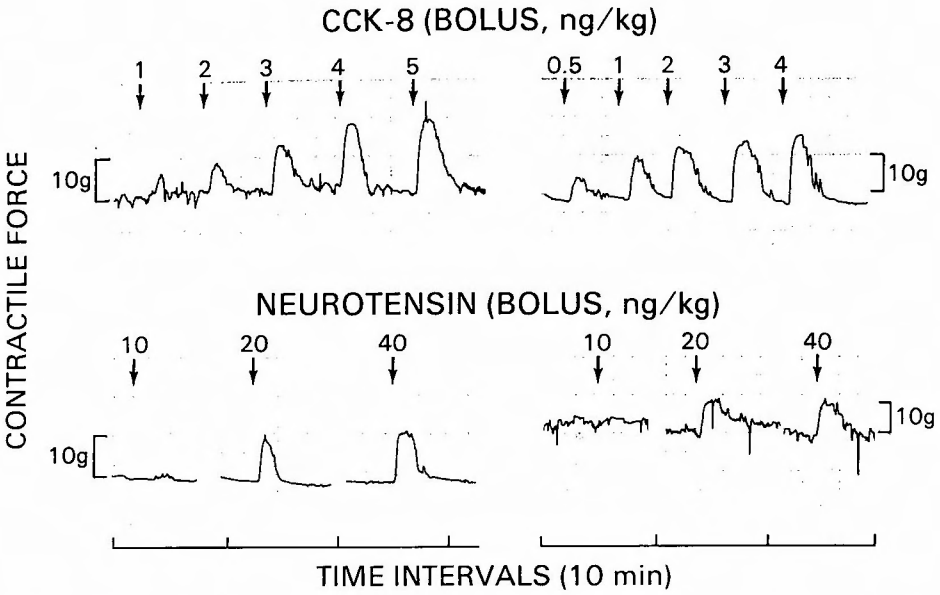

Fig. 1. Effects of a bolus intravenous injection of CCK-8 (upper panel) and NT (lower panel) recorded from two different dogs.

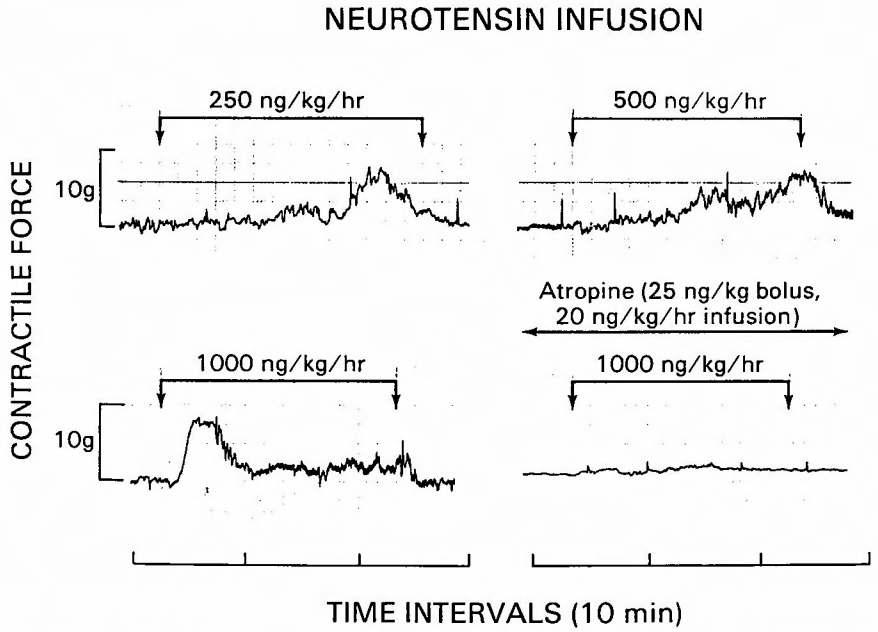

Fig. 2. Effects of continuous intravenous infusion of NT at doses of 250, 500 and 1000ng/kg/hr (upper panels and lower left panel), and at a dose of 1000ng/kg/hr under the treatment of atropine (25 ng/kg bolus followed by 20 ng/kg/hr infusion) (lower right panel).

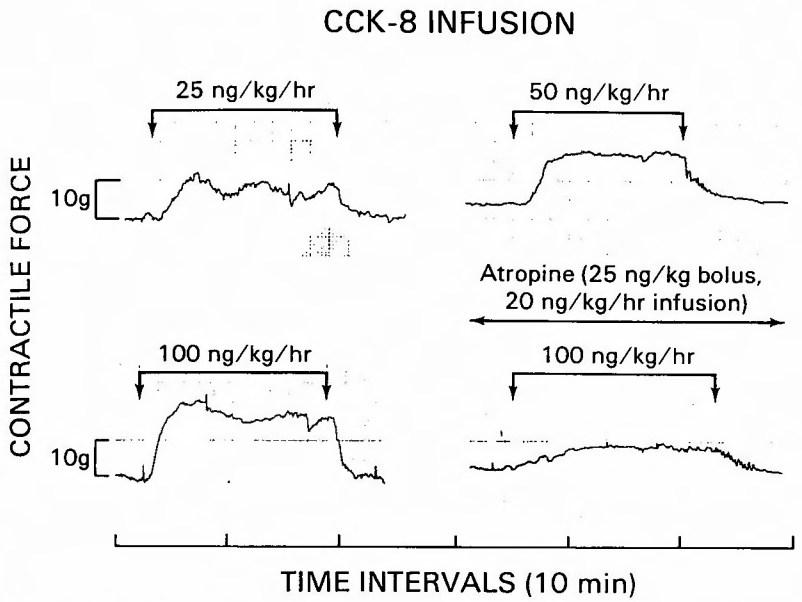

Fig. 3. Effects of continuous intravenous infusion of CCK-8 at doses of 25, 50 and 100ng/kg/hr (upper panels and lower left panel), and at a dose of 100ng/kg/hr under the treatment of atropine (25ng/kg bolus followed by 20ng/kg/hr infusion) (lower right panel).

kg/hr to 500 ng/kg/hr. Increasing the dose of CCK-8 from 25 ng/kg/hr to 50 ng/kg/hr significantly increased the contractile force of the gallbladder and significantly decreased the onset time of gallbladder contraction. Contractile force at maximum concentration obtained by CCK-8 at doses of 25 and 50 ng/kg/hr was significantly larger and onset time of contraction was significantly shorter than those obtained by NT at 250 or 500 ng/kg/hr.

In vitro study

Tension in gallbladder strips increased uniformly in response to CCK-8 but NT had no effects in this system (data not shown).

Table 1. Summarized data of effects of NT and CCK-8 on contractile force at maximum contraction (gram) and onset time of contraction (minutes). *=P<0.05 versus NT 250 or 500ng/kg/hr △=P<0.05 versus CCK-8 25ng/kg/hr.

Peptide	Dose (ng/kg/hr)	n	Contractile Force at Maximum Contraction (g)	Onset Time of Contraction (min)
NT	250	5	6.6±1.2	6.2±0.6
NT	500	5	7.6±0.4	5.8±1.1
CCK-8	25	5	9.3±0.8*	2.4±0.4*
CCK-8	50	5	12.5±0.7*△	1.5±0.3*△

Discussion

The present study shows that NT, a gut peptide, stimulates gallbladder contraction in the dog in vivo. Contractile effects of NT were transient and were not sustained during the continuous infusion of NT. The magnitude of contractile force was not dose-dependent. Furthermore, atropine completely abolished the stimulatory effect of NT on gallbladder contraction. These data, together with the in vitro study, in which NT had no effect on rabbit gallbladder strips, indicate that the action of NT on the gallbladder is apparently mediated by cholinergic pathways.

CCK, a well established gut peptide, causes gallbladder contraction[2,40] by a direct action at the gallbladder muscle[37,43]. CCK-8 was used as a control not only to compare with NT but also to insure the accuracy of the methods engaged in this study. Thus, as it was reported[39], gallbladder contractions induced by CCK-8 in this study were found to cause dose-dependent contractions which were tonic and remained at a constant level as long as CCK-8 was infused intravenously. Atropine diminished gallbladder contractions induced by CCK-8. Myoelectric activity of the duodenum was monitored by a bipolar electrode in the duodenal muscle to avoid periodic contractions of the gallbladder which occur in close association with the interdigestive immigrating contraction[20].

In the present study, bolus injection of different doses of NT did not change the magnitude of contractile force. SAKAMOTO et al.[33] reported that IV bolus injection of NT caused an elevation of gallbladder pressure and atropine decreased gallbladder pressure induced by NT in chronic dogs with gallbladder fistulas. They also reported that NT increased gallbladder pressure in a dose-dependent manner and NT was approximately 1 : 50 as potent as CCK-8, on a molar basis. In direct measurements of muslce contraction, NT-induced gallbladder contractions were not altered by different doses of NT. This might be due to the different experimental methods or to gallbladder pressure which is affected by many factors, such as sphincter of ODDI, cystic duct and so on.

Furthermore, previous studies also made use of pharmacologic doses of NT, since they used large amounts of NT (doses of 0 25, 0.5, 1.0 and 2.0 uμ/kg as a bolus injection were tested). The doses of NT used in this study (as a bolus injection) were much less, but these doses are still considered pharmacological since we did not determine whether blood levels of circulating NT obtained by bolus injection of NT exceeded physiological blood levels of NT. On the other hand, continuous IV infusions of NT, at doses of 250 and 500 ng/kg/hr, were considered to be physiologic, since blood levels of NT obtained by these doses were comparable to those achieved by endogenously released NT in response to intraduodenal perfusion of Lipomul at a dose of 2 g/kg/hr[34].

NT-induced gallbladder contractions in vitro were not observed, indicating that the action of NT on the gallbladder is not directly at the gallbladder muscle. However, it might exert its actions at sites remote from the gallbladder. The same observation was reported for motilin which caused a dose-dependent contraction of the gallbladder in conscious pigs[1]. However,

motilin had no effect on the gallbladder muscle in vitro[38]. Pancreatic polypeptide which caused gallbladder relaxation[16] in vivo did not have any effects in vitro[28].

Surprisingly, infusion of NT in normal human volunteers caused gallbladder relaxation assessed by ultrasonography[41]. The mechanism of this effect is not known. It seems difficult to attribute this to species differences. NT can release PP[6,7,24], which can relax the gallbladder, or NT can affect norepinephrine which inhibits both vagal and field-stimulated responses by actions on α_2-adrenergic receptors on pre- and post-ganglionic cholinergic neurons[13]. Thus, further studies are needed to understand better the effects of NT on gallbladder motility.

Acknowledgments

This work was supported by grants from the National Institutes of Health (R01 DK 15421, and P01 DK 35608). The author wishes to express his sincere gratitude to Associate Professor GEORGE H GREELEY Jr., Professor JAMES C. THOMPSON, Department of Surgery, University of Texas Medical Branch, Galveston, Texas, U.S.A. and Professor TAKAYOSHI TOBE, Department of Surgery, Faculty of Medicine, Kyoto University, Kyoto, Japan for their great support, and Drs. TSUGUO SAKAMOTO, Department of Surgery, Osaka University School of Medicine, Osaka, Japan and FELIX LLUIS, Department of Surgery, Universidad Autonoma de Barcelona, Barcelona, Spain for their exceptional help.

References

1) Adrian TE, Michenere P, Sagor GR, et al: Effect of motilin and other gut hormones on gallbladder pressure. Regul Pept **1** (Suppl 1): S1, 1980.
2) Amer MS: Studies with cholecystokinin. II. Cholecystokinetic potency of porcine gastrins I and II and related peptides in three systems. Endocrinology **84**: 1277, 1969.
3) Andersson S, Chang D, Folkers K, et al: Inhibition of gastric acid secretion in dogs by neurotensin. Life Sci **19**: 367, 1976.
4) Berry H, Flower RJ: The assay of endogenous cholecystokinin and factors influencing its release in the dog and cat. Gastroenterology **60**: 409, 1971.
5) Blackburn AM, Bloom SR, Long RG, et al: Effect of neurotensin on gastric function in man. Lancet **1**: 987, 1980.
6) Blackburn AM, Fletcher DR, Adrian TE, et al: Neurotensin infusion in man: Pharmacokinetics and effect on gastrointestinal and pituitary hormones. J Clin Endocrinol Metab **51**: 1257, 1980.
7) Blackburn AM, Bloom SR, Edwards AV: Pancreatic endocrine responses to exogenous neurotensin in the conscious calf. J Physiology **314**: 11, 1981.
8) Carraway RE, Leeman SE: The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. J Biol Chem **248**: 6854, 1973.
9) Carraway RE, Leeman SE: The amino acid sequence of a hypothalamic peptide, neurotensin. J Biol Chem **250**: 1907, 1975.
10) Carraway RE, Leeman SE: The synthesis of neurotensin. J Biol Chem **250**: 1912, 1975.
11) Carraway RE, Leeman SE: Radioimmunoassay for neurotensin, a hypothalamic peptide. J Biol Chem **251**: 7035, 1976.
12) Carraway RE, Leeman SE: Characterization of radioimmunoassayable neurotensin in the rat: Its differential distribution in the central nervous system, small intestine and stomach. J Biol Chem **251**: 7045, 1976.
13) Daniel EE, Sakai Y, Jury J, et al: Mode of action of neurotensin on gastrointestinal motility, in Wienbeck M (ed): Motility of the Digestive Tract. New York, Raven Press, 1982, P451.

14) Doyle H, Greeley GH Jr, Mate L, et al: Distribution of neurotensin in the canine gastrointestinal tract. Surgery **97**: 337, 1985.
15) Frigerio B, Ravazola M, Itoh S, et al: Histochemical and ultrastructural identification of neurotensin cells in the dog ileum. Histochemistry **54**: 123, 1977.
16) Greenberg GR, Adrian TE, Baron JH, Mccloy RF, et al: Inhibition of pancreas and gallbladder by pancreatic polypeptide. Lancet **2**: 1280, 1978.
17) Helmstaedter V, Feurle GE, Forssmann WG: Ultrastructural identification of a new cell type—the N-cell as the source of neurotensin in the gut mucosa. Cell Tissue Res **184**: 445, 1977.
18) Itoh Z, Honda R, Takeuchi S, et al: An extraluminal force transducer for recording contractile activity of the gastrointestinal smooth muscle in the conscious dogs: Its construction and implantation. Gastroenterol Jpn **12**: 275, 1977.
19) Itoh Z, Takeuchi S, Aizawa I, et al: Characteristic motor activity of the gastrointestinal tract in fasted conscious dogs measured by implanted force transducers. Dig Dis **23**: 229, 1978.
20) Itoh Z, Takahashi I: Periodic contractions of the canine gallbladder during the interdigestive state. Am J Physiol **240**: G183, 1981.
21) Iwasaki Y, Itoh S, Shibata A: Radioimmunoassay of neurotensin and the distribution and concentration of gut neurotensin in rat and dog. Tohoku J Exp Med **130**: 129, 1980.
22) Khalil T, Fujimura M, Greeley GH Jr, et al: Neurotensin stimulates pancreatic exocrine secretion in rats. Regul Pept **15**: 279, 1986.
23) Lonovics J, Guzman S, Devitt P, et al: Release of pancreatic polypeptide in human by infusion of cholecystokinin. Gastroenterology **79**: 817, 1980.
24) Newman J, Townsend CM Jr, Greeley GH Jr, et al: Neurotensin: Effect on pancreatic secretion and release of gastrin, pancreatic polypeptide, and cholecystokinin-33 in dogs. Surg Forum **34**: 213, 1983.
25) Olsen PS, Pedersen JH, Kirkegaard P, et al: Neurotensin induced inhibition of gastric acid secretion in duodenal ulcer patients before and after parietal cell vagotomy. Gut **25**: 481, 1984.
26) Orci L, Baetens O, Rufener C, et al: Evidence for immunoreactive neuroten in in dog intestinal mucosa. Life Sci **19**: 559, 1976.
27) Polak JM, Bloom SR: Neuropeptides of the gut: A newly discovered major control system. World J Surg **3**: 393, 1979.
28) Pomeranz IS, Davison JS, Shaffer EA: In vitro effects of pancreatic polypeptide and motilin on contractility of human gallbladder. Dig Dis Sci **28**: 539, 1983.
29) Read NW, McFarlane A, Kinsman RI, et al: Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon. Gastroenterology **86**: 274, 1984.
30) Rosell S, Burcher E, Chang D, et al: Cardiovascular and metabolic actions of neurotensin and (glu^4)-neurotensin. Acta Physiol Scand **98**: 484, 1976.
31) Rosell S, Rokaeus A: The effect of ingestion of amino acids, glucose and fat on circulating neurotensinlike immunoreactivity (NTLI) in man. Acta Physiol Scand **107**: 263, 1979.
32) Rosell S, Al-Saffar A, Thor K: The role of neurotensin in gut motility, Scand J Gastroenterol **19** (Suppl. 96): 69, 1984.
33) Sakamoto T, Mate L, Greeley GH Jr, et al: Effect of neurotensin on gallbladder contraction in dogs. Gastroenterology **86**: 1229, 1984.
34) Sakamoto T, Newman J, Townsend CM, et al: Role of neurotensin in the intestinal phase of pancreatic polypeptide release induced by fat. Gastroenterology **86**: 1229, 1984.
35) Sakamoto T, Newman J, Fujimura M, et al: Role of neurotensin in pancreatic secretion. Surgery **96**: 146, 1984.
36) Sakamoto T, Fujimura M, Townsend CM Jr, et al: Interaction of neurotensin, secretin and cholecystokinin on pancreatic exocrine secretion in conscious dogs. Surg Gyn Obst **166**: 11, 1988.
37) Steigerwalt W, Goldfine ID, Williams JA: Characterization of cholecystokinin receptors on bovine gallbladder membranes. Am J Physiol **247**: G709, 1984.
38) Strunz U, Domschke W, Mitznegg P, et al: Analysis of the motor effects of 13-norleucine motilin on the rabbit, guinea pig, rat and human alimentary tract in vitro. Gastorenterology **68**: 1485, 1975.
39) Takahashi I, Suzuki T, Aizawa I, et al: Comparison of gallbladder contractions induced by motilin and cholecystokinin in dogs. Gastroenterology **82**: 419, 1982.
40) Vagne M, Grossman MI: Cholecystokinetic potency of gastrointestinal hormones and related peptides. Am

J Physiol **215**: 881, 1968.
41) Walker JP, Khalil T, Wiener I, et al: The role of neurotensin in human gallbladder motility. Ann Surg **201**: 678, 1985.
42) Yanaihara N, Sato H, Inoue A, et al: Comparative study on distribution of bombesin-, neurotensin- and β-endorphin-like immunoreactivities in canine tissues. Adv Exp Med Biol **120**A: 29, 1979.
43) Yau WM, Makhlouf GM, Edwards LE, et al: Mode of action of cholecystokinin and related peptides on gallbladder muscle. Gastroenterology **65**: 451, 1973.

和文抄録

Neurotensinに関する研究
Ⅰ. 胆嚢運動について

滋賀医科大学外科学第2講座
藤　村　昌　樹

消化管ホルモンである neurotensin (NT) の胆嚢運動に及ぼす作用を in vivo および in vitro で検討した．実験には，胆嚢平滑筋に直接収縮作用をもつ消化管ホモンである cholecystokinin-8 (CCK-8) をコントロールとして用いた．In vivo での実験では，5頭の雑種成犬の胆嚢に force transducer を縫着し胆嚢運動を生理的条件下で記録した．NT (20 および 40 ng/kg) の急速静注により胆嚢収縮がみとめられたが収縮の強さは投与量により変化しなかった．CCK-8 の急速静注では，用量依存性に胆嚢収縮が発現した．また，NT (250 および 500 ng/kg/hr) の持続注でも胆嚢収縮は惹起された．これらの投与量での NT の血中濃度は，脂肪による NT の内因性放出時の血中濃度に匹敵するものであり，生理的範囲内の投与量であった．従って，この胆嚢収縮は NT の生理的作用とみなすことができる．しかしながら NT の血中への持続投与による胆嚢収縮は一時的で，投与中でも収縮は持続しなった．また投与量の違いによっても最大収縮の強さ，および収縮運動発現までの時間に差はみられなかった．アトロピンの投与下では NT による胆嚢収縮はまったくみられなかった．それに対して CCK-8 の胆嚢収縮作用は静脈内投与中ほぼ一定して持続し，この作用には用量依存性の収縮力の増大と，作用発現時間の短縮がみられた．一方，ウサギ胆嚢筋条片を用いた in vitro での実験では，NT の作用はみられなかったが，CCK-8 は用量依存性に胆嚢筋条件を収縮させた．

以上の結果から，NT にはその生理的作用の一つとしてイヌ胆嚢の収縮に働くこと，そしてこの作用は迷走神経を介していることが明らかとなった．